Phase Transformations During Freeze-Drying: Potential Implications on Drug Product Performance

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Pharmaceutical freeze-drying

Prelyo solution

Amorphous

Glass transition ($T_g'$)

Melt-back

Crystalline

Eutectic ($Te$)

Drying

Cake collapse

$T > T_g'$
In situ phase transition

• Monitoring them can be a challenge but valuable

• Multiple analytical techniques may be needed

• Excipients – use judiciously; more is NOT better

• Potential for interaction between formulation components
  – Influence the physical form
## Function-specific solid-state

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Common examples</th>
<th>Desired solid-state</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small molecules</td>
<td>Antibiotics, oncolytics</td>
<td>Crystalline</td>
</tr>
<tr>
<td>Proteins</td>
<td>--</td>
<td>Difficult to crystallize</td>
</tr>
<tr>
<td>Bulking agents</td>
<td>Mannitol, glycine</td>
<td>Crystalline</td>
</tr>
<tr>
<td>Buffers</td>
<td>Phosphate, histidine, citrate</td>
<td>Amorphous</td>
</tr>
<tr>
<td>Lyoprotectants</td>
<td>Sucrose, trehalose</td>
<td>Amorphous</td>
</tr>
</tbody>
</table>
Case studies

• Trehalose crystallization
• Mannitol hemihydrate
• In situ salt formation
• Salt disproportionation
Protein stabilization

Lyoprotectant hydrogen bonds to protein – prevents denaturation

Lyoprotectant crystallizes

Ability to hydrogen bond with protein?

Protein stable?

Conventional wisdom
Kapton® film window
Aluminum holder
Frozen solution or lyophile
Connection to cooling system
X-ray beam Detector

Varshney et al, Pharm Res, 2009

Cell dimensions:
A = 10 mm, B = 12 mm, C = 8 mm, D (thickness) = 2 mm
2-Dimensional XRD using high intensity sources

Transmission geometry

X-ray source

Sample

2-D X-ray pattern of crystalline sucrose

C. Nunes, Ph.D. Dissertation, Univ of MN
Trehalose frozen solution

4% Trehalose

Evidence of crystallization of trehalose dihydrate in frozen solutions

Why was trehalose crystallization not reported in the literature?
Phase transition during drying

Sundaramurthi and Suryanarayanan, J Phys Chem Lett, 2010
Frozen solution

\[(\text{C}_{12}\text{H}_{22}\text{O}_{11}\cdot2\text{H}_{2}\text{O})\]

Crystalline

drying

Lyophilic

\[(\text{C}_{12}\text{H}_{22}\text{O}_{11})\]

Amorphous

Dehydration

Crystalline → amorphous transition
Trehalose & mannitol frozen solution

Trehalose (4% w/v)
Mannitol (2% w/v)

Annealed at -18 °C (h)

Intensity (arbitrary units)

2θ (°)

Sundaramurthi and Suryanarayanan, Pharm Res 27 (2010) 2384
Role of the protein?
Effect of proteins

Trehalose (4% w/v)
mannitol (2% w/v)
LDH (2 mg/ml)

Lactic dehydrogenase (LDH)

* Trehalose dihydrate ( )
# Mannitol hemihydrate ( )

Annealed at -18 °C (h)

Intensity (arbitrary units)

2θ (°)

Sundaramurthi and Suryanarayanan, Pharm Res, 27 (2010) 2384
• Protein inhibits trehalose crystallization

• This effect is concentration dependent
Sequence of events

Protein inhibits trehalose crystallization

Trehalose is retained amorphous

Amorphous trehalose functions as an effective lyoprotectant and stabilizes the protein
Case studies

• Trehalose crystallization
• Mannitol hemihydrate
• In situ salt formation
• Salt disproportionation
Mannitol

- Bulking agent

- Advantages
  - Readily crystallizes
  - High eutectic temperature

- Potential issue
  - Formation of mannitol hemihydrate (MHH) during lyophilization

Mehta et al, Eur J Pharm Biopharm 85 (2013) 207
MHH – effect on product stability

Stoichiometric water content - 4.7% w/w

Dehydration and Release of water during storage

• API – hydrolysis
• Moisture-induced protein aggregation
• Plasticize amorphous components – crystallization. For example – sucrose*

*Bhatnagar et al, unpublished work
Once formed, MHH is difficult to dehydrate

50 °C

**Colyophilized MHH - sucrose**
Extremely slow dehydration

**Pure MHH**
Rapid dehydration
Strategy: Avoid MHH formation during lyophilization.

How?
Mannitol phase formed – appeared to depend on the temperature of crystallization.

Aq. mannitol solution (10% w/v)

Intensity, arbitrary counts

2θ, degrees
Hypothesis - The temperature of crystallization (during cooling) governs the mannitol phase crystallizing from solution.
Frozen state characterization

Synchrotron XRD – Argonne National Labs

• Real time monitoring during freezing

• High sensitivity
MHH first forms during the freezing step.
Prevent MHH formation in lyo product by modifying cycle using ControLyo™ technology

Ice nucleation at -5 °C

Primary drying
No MHH in the final lyo product

Characteristic MHH peak ($18.1^\circ 2\theta$) absent

Mannitol solutions

Mannitol – sucrose solutions
Controlling the physical form of mannitol..

- Temperature of mannitol crystallization
  - Governed by the ice crystallization temperature

Monitoring the frozen solution
Case studies

• Trehalose crystallization
• Mannitol hemihydrate
• In situ salt formation
• Salt disproportionation
Sequence of events during freeze drying

- Ice formation
- Freeze concentrate
- Increased interaction between solutes in the concentrate
  - Multicomponent formulation
    - Solute crystallization?
    - Formation of new species?
  - Primary and secondary drying
    - Freeze dried solid
Ions in solution

Crystalline salt

Amorphous salt

Ions in freeze concentrate

Annealing

Freeze drying
Model system

Indomethacin (IMC) with tris as counter ion

0.1 M IMC in 0.15 M tris

pH of the final solution ~7.7
IMC (0.1 M)  
pKa = 4.0  
Complete ionization

Tris (0.15 M)  
pKa = 8.0  
~65% ionized  
(\equiv 0.0975 M)

Solution pH = ~7.7

Formation of IMC-tris salt
IR spectra

- **IMC-tris lyophile**
- **Tris**
- **Amorphous IMC**

- **Loss of free/hydrogen bonded COOH in salt**
- **Asymmetric COO⁻ stretch in salt**
- **Amine salt symm NH₃⁺ 1550-1505 cm⁻¹**
X-ray powder diffraction patterns

- IMC (crystalline)
- Tris (freeze dried)
- Lyophile (without annealing)
- Lyophile (with annealing)
Vials loaded in lyophilizer

Before annealing (frozen)

After annealing (frozen)
Dissolution profiles

- Crystalline IMC
- Physical mixture
- Lyophile without annealing
- Lyophile with annealing

Time (minutes)

Fraction of drug dissolved

0 10 20 30 40 50 60 70 80 90

0.0 0.2 0.4 0.6 0.8 1.0 1.2
Summary

• Salt formation during freeze-drying

• Enhanced dissolution

• Annealing - control the physical form of the lyophile
Case studies

• Trehalose crystallization
• Mannitol hemihydrate
• In situ salt formation
• Salt disproportionation
Buffer Crystallization - Schematic

Sodium phosphate buffer

\[ \text{acid} + \text{salt of acid} \rightarrow \text{buffer} \]

\[ \text{NaH}_2\text{PO}_4 + \text{Na}_2\text{HPO}_4 \rightarrow \text{pH 7.4} \]

\[ \text{pH 3.5} \]
Working Hypothesis

In indomethacin sodium/sodium phosphate buffer system

Selective crystallization of buffer component and the consequent pH shift causes disproportionation of indomethacin sodium salt resulting in formation of poorly soluble indomethacin free acid.

Koranne et al, unpublished results
Buffer crystallization & pH shift

Prelyophilization solution → Cool → Ice crystallization & freeze concentration → Buffer salt crystallization → pH shift → Disproportionation → Salt → Free acid

Model system
Indomethacin sodium (IMCNa)

pH shift

pH < pH_{\text{max}}

IMCNa

IMC free acid
FTIR IMC free acid

FTIR IMCNa salt

1735
1717
1710
1735

1560
1589

IMCNa. $3\text{H}_2\text{O}$

amorphous IMCNa
Lyophiles: FTIR Summary

<table>
<thead>
<tr>
<th>NaP concentration, mM</th>
<th>IMCNa trihydrate concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 mg/ml</td>
</tr>
<tr>
<td></td>
<td>10 mg/ml</td>
</tr>
<tr>
<td></td>
<td>5 mg/ml</td>
</tr>
<tr>
<td></td>
<td>(34.6 mM)</td>
</tr>
<tr>
<td></td>
<td>(23 mM)</td>
</tr>
<tr>
<td></td>
<td>(11.5 mM)</td>
</tr>
<tr>
<td>100</td>
<td>D</td>
</tr>
<tr>
<td>50</td>
<td>D</td>
</tr>
<tr>
<td>35</td>
<td>ND</td>
</tr>
<tr>
<td>20</td>
<td>ND</td>
</tr>
<tr>
<td>10</td>
<td>ND</td>
</tr>
</tbody>
</table>

D: Disproportionation (IMC acid formation) observed
ND: No disproportionation (no IMC acid formation) observed
Summary: Low temperature pH measurements

<table>
<thead>
<tr>
<th>NaP concentration, mM</th>
<th>IMCNa ( \cdot 3\text{H}_2\text{O} ) concentration, mg/ml (mM)</th>
<th>pH at 20 °C(a)</th>
<th>pH at -25 °C(b)</th>
<th>ΔpH(c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>10 (23.0)</td>
<td>7.1</td>
<td>2.9</td>
<td>4.2</td>
</tr>
<tr>
<td>100</td>
<td>-</td>
<td>7.1</td>
<td>2.8</td>
<td>4.3</td>
</tr>
<tr>
<td>35</td>
<td>-</td>
<td>7.2</td>
<td>3.1</td>
<td>4.1</td>
</tr>
<tr>
<td>35</td>
<td>15 (11.5)</td>
<td>7.2</td>
<td>6.7</td>
<td>0.5</td>
</tr>
<tr>
<td>35</td>
<td>10 (23.0)</td>
<td>7.2</td>
<td>5.2</td>
<td>2.0</td>
</tr>
<tr>
<td>35</td>
<td>5 (34.6)</td>
<td>7.1</td>
<td>5.6</td>
<td>1.5</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>7.2</td>
<td>3.5</td>
<td>3.7</td>
</tr>
<tr>
<td>10</td>
<td>10 (23.0)</td>
<td>7.2</td>
<td>7.4</td>
<td>-0.2</td>
</tr>
</tbody>
</table>

Initial pH (a)
pH after the buffer solution was cooled to -25 °C and held for 2 hours (b)

\[ \Delta p\text{H}^{(c)} = p\text{H}^{(a)} - p\text{H}^{(b)} \]

Maximum error in pH measurements is ±0.1

- pH shift during freezing causes disproportionation
DSC - Prelyophilization Solutions

- Absence of IMCNa crystallization exotherm in systems that undergo disproportionation.
Low temperature XRD - Prelyophilization Solutions

Dibasic sodium phosphate dodecahydrate

Hexagonal ice peaks

Intensity (arbitrary counts)

2θ (°)

- 5 mg/ml-35mM
- 10 mg/ml-35mM
- 15 mg/ml-35mM
- 35 mM buffer

Dibasic sodium phosphate dodecahydrate

Hexagonal ice peaks
Conclusions

• Disproportionation of IMCNa due to selective crystallization of buffer component (\(\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}\)) and the consequent pH shift

• Disproportionation is dependent on concentration of buffer and IMCNa

• The absence of IMCNa crystallization event in DSC heating curves indicates disproportionation
Summary

• Selection of excipient
  – Product stability hinges on excipient functionality
  – Physical form of the excipient can be critical

• Excipient concentration – select judiciously
  – More is NOT better

• Excipient with multiple functionalities
  – Has the potential to simplify the formulation
Summary

• Complex interplay of drug and excipients
  – API can influence excipient behavior
  – One excipient can influence the behavior of a second excipient

• Numerous processing steps
  – Potential for phase transitions
  – Monitoring them can be a challenge but very valuable
    • Multiple analytical techniques may be needed